Tissue Engineering Literature Review

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Literature Review

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Tissue Engineering

Tissue injury is a broad term that accounts for many hospital visits annually. These injuries consist of two main types: autonomously reparable injuries, such as dermal and bone injuries, and non-autonomously reparable injuries, such as neural and cartilage injuries. Although autonomously reparable tissues have increased regeneration rates, some cases of severe damage can result in a permanent state of irreparability. For both severe tissue damage and non-autonomously reparable tissue damage, the most widely used method of repairing these tissues is transplants. There are 3 main categories of transplant tissue: autografts, allografts and xenografts. Autografts are tissues that are taken from the patient's own body for transplants. While autografting has advantages, such as high biocompatibility, autografts can be very difficult to obtain, as much of the tissue in a person's body is constantly being used for essential bodily functions. On the other hand, the supply of tissue for allografts, transplantation from human tissue donors, and xenografts, transplantation of animal tissues, is more plentiful, but biocompatibility is lower (Ikada 2006). Tissue engineering, a developing field in biomedical engineering, uses surrounding tissue to grow back the damaged tissue that would have been irreparable otherwise. The process primarily revolves around three integral components: seeded cells, growth factors and cellular scaffolds. Seeded cells are the cells that the body uses to repeatedly divide and make other cells, creating a uniform tissue. Growth factors are used to help the seeded cells develop and mature until the cells are ready to divide. Finally, cellular scaffolds are the framework for the cells to grow on. Cellular scaffolds have many different properties that make them unique and are a major component in tissue engineering. The purpose of these scaffolds is to model natural extracellular matrices. Extracellular matrices have five main functions: to provide structural support for cells to grow on, to give the tissue its rigidity and flexibility for its basic processes, provide the cues for specific cell functions, to act as reservoirs for growth factors, and provide a degradable environment for morphological changes (Chan 2008).

Mechanical Properties

The mechanical properties of a scaffold are very important in making decisions for whether or not certain scaffolds should be used for tissue engineering. In soft tissue engineering, rigid scaffolds should not be used because they limit movement. Likewise, soft scaffolds would not be suitable for rigid tissue engineering because of the limited compression stress they can withstand without breaking. Generally, synthetic scaffolds with similar mechanical properties as their naturally occurring tissue work best for tissue engineering. As a result, the focus of tissue engineering has gone towards trying to make the mechanical properties of synthesized scaffolds similar to naturally produced extracellular matrices. Currently, there is no perfect method of controlling the mechanical properties of these scaffolds. However, different combinations of biomaterials and methods of synthesis make it possible to create synthetic scaffolds with similar mechanical properties to those of natural extracellular matrices.

There are six main ways of categorizing scaffolds based on their mechanical properties. The first, external geometry, defines the shape of the scaffold, governing aspects such as the physical area of regeneration. The second, surface properties, assist the movement of extracellular molecules. The third, pore size, controls the size of cells that are able to grow in the scaffold, limiting cells from growing too big or staying too small. The fourth, interface adherence, controls how well the scaffold sits in its environment. The fifth, degradation character, regulates how easily the scaffold degrades once the cells grow back. The sixth and final important mechanical characteristic for all scaffolds is the mechanical competence of the scaffold, which includes mechanical properties such as elastic modulus and tensile strength (Bharatheeswaran 2011). When deciding whether to use a scaffold for medical purposes, these six properties are tested, compared to natural tissue samples, and a decision is made on whether to use the scaffold.

Elastic Modulus

One of the most important mechanical properties of a material is its elastic modulus. Elastic modulus is defined as an object's ability to resist physical deformations. In tissue engineering, the elastic modulus is one of the most important properties of the scaffold, and is a deciding factor in whether or not a synthesized scaffold should be used in a certain situation. As stated before, soft tissue engineering requires a relatively high elasticity in order to maintain dynamic movement, while hard tissue engineering requires low elasticity, in order to maintain rigidity (Diekman 2012). The elastic modulus of a material can generally be calculated by finding the slope of the stress versus strain curve. The stress that an object experiences is defined as the force being applied on that object, while the strain of the object is defined as the change in the ratio of some parameter, such as length, when a certain stress is applied.

In addition, there are three main different types of elastic moduli- Young's modulus, shear modulus and the bulk modulus. The first, Young's modulus, describes tensile elasticity. This measures the elasticity when an object is deformed on a single axis by opposing forces. The shear modulus is similar to Young's modulus in that the force deforms the object into a constant volume, but not necessarily on the same axis. Finally, the bulk modulus is the tendency of an object to deform in all different directions, therefore changing the volume of the object. The mechanical properties of a biomaterial govern how suitable it is for tissue engineering.

Flexural Modulus

The flexural modulus of an object is defined as the object's tendency to bend. It can also be described as the slope of the stress versus strain graph when discussing flexural deformation. Flexural modulus can be calculated using a method called a three point test, where two ends of an object are placed on supports and a force is applied to the center of the object. The flexural modulus of biomaterials is important for engineering highly flexible places, such as joints. Joints typically require high flexural moduli, as there is almost constantly a three point force being applied (ASTM 2003).

Compressional Strength

The compressional strength of a material is its ability to withstand compressive forces. Every object has an intrinsic compression force limit. Past that limit, the material either breaks or deforms irreversibly. This limiting force is a key feature when synthesizing scaffolds. When dealing with tissue in a high compression environment, such as joints, the scaffold must have a fairly high compressive strength. For example, if a very rigid scaffold with a low compressive limit is used to regenerate a tissue that experiences lots of compressive force, the scaffold has a risk of cracking under potential mechanical stress. Compressional forces are generally used when dealing with malleable materials, as brittle materials generally cannot withstand much compressive force. Their compressive limits are nearly negligible when compared to a malleable material. Therefore, instead of compressional strength, the tensile strength is used to measure the mechanical properties of a brittle material. The compressional strength of an object is a key property used for determining how fit a scaffold is for soft tissue engineering (AZoM, 2016).

Tensile Strength

The tensile strength of a material is its ability to withstand tension forces, as opposed to compressional strength, which is the ability of a material to withstand compressional forces. Tensile strength is commonly used in the analysis of brittle materials because, for brittle materials, the compressional forces are on a significantly lower magnitude compared to malleable materials. Soft tissues are not suitable for tensile strength tests, as they end up ripping due to the tensional forces. These tension force limits greatly affect which synthesized scaffold should be used for the regeneration of certain tissues. For example, in joints, areas that extend more should have a high tension force limit. These forces can rise up to values as high as 170 kg/m^3 (Bullough, 1970). If a high tension force limit is not used, the tissues in that area will rip, leading to more tissue damage in the body. (NDT n.d.)

Average Pore Size

The pore sizes of the scaffolds greatly affect the regeneration rate of the cells inside the scaffold as well. The porosity in the scaffold is arguably the most important feature that a scaffold has. Without a high porosity, there would be no interconnected pathways for cell growth. However, with a high pore density, the scaffold becomes too weak, brittle and unfit for application. The pore size largely determines how slow or fast the cells grow in the scaffold. The pores in the scaffold must be able to comfortably fit the cells inside, without restricting their growth, but at the same time must be small enough to be able to hold the cells in place. Pore size can be measured in many ways, the most common of which is simply looking at the synthesized scaffold under a microscope and estimating the size (Chan, 2008). Pore size is a very important property that governs how cells grow inside a scaffold.

Biomaterials

The biomaterials that compose cellular scaffolds largely define the mechanical properties of the scaffolds. The biomaterial must have a few key properties in order to regenerate damaged tissue without causing other bodily harm. Firstly, the materials must be biocompatible. Tissue engineering is favored over transplants mainly because of biocompatibility, as transplants may cause the body to reject foreign tissue. Similarly, in tissue engineering the scaffold must be biocompatible, otherwise the body would reject the synthesized scaffold in the same way it would reject transplanted tissue. However, biocompatibility of materials is much easier to achieve than biocompatibility of entire tissues. Another important feature required is biodegradability. This feature is important because once the cells grow back in the scaffold, the structure must break down to save space for the natural extracellular matrix to grow. If the scaffold is non-bioresorbable, the cells could get crushed by the residual scaffold. The final aspect that scaffolds must have is a similar set of mechanical properties as a natural extracellular matrix of normal tissue. For example, if the damaged tissue was flexible before damage, a rigid scaffold would lead to reduced mobility. Similarly, in an area of hard tissue growth, such as bone tissue, a soft scaffold could lead to possible damage (O'Brien 2011).

There are two main classes of biomaterials used to make scaffolds: natural polymers and synthetic polymers. The first, natural polymers, are derived from natural sources. This group of biomaterials consists of collagen, elastin and other materials found in natural extracellular matrices. Natural polymers generally have high biocompatibility and biodegradability, but the mechanical properties of these materials are hard to predict. On the other hand, synthetic polymers, such as polylactic acid (PLA) and phosphoglyceric acid (PGA), can be crafted such that they express the desired mechanical properties, but they are rarely biocompatible and biodegradable (Nigam 2014). Because of this duality in the properties of the biomaterials, a mixture of synthetic and natural polymers is used in medical practice (Chan 2008).

Silk proteins

Soft tissue engineering is one of the most focused areas of tissue engineering study. Damaged soft tissue generally autonomously regenerates at much slower rates than hard tissues. Take neural tissue for example. The regeneration rate of neural tissue is so long that when damaged, neural tissue may not regenerate in an entire lifetime. Another difficulty of soft tissue engineering is the flexibility that the synthesized scaffold must have in order to keep the growing tissue flexible. Silk proteins have emerged as versatile and cheap biomaterials that can be used for tissue engineering, as they have many properties that make them favorable in tissue engineering. These natural proteins are generally very compatible with cells, reducing the risk of the host body rejecting the scaffold. Also, for the same reasons, silk is biodegradable- another advantage of using it for tissue engineering.

Many methods of synthesis have been used to make silk scaffolds, such as solvent casting and particulate leaching, gas foaming, and lyophilization (freeze drying). The most common of these is solvent casting and particulate leaching (SCPL), because it creates a sturdy scaffold suitable for bone tissue engineering. However, for soft tissue engineering, lyophilization techniques become a much better option than solvent casting and particulate leaching. Silk lyophilization techniques make it easy to control the mechanical properties of the synthesized scaffold. Various alterable properties of silk proteins allow for the mechanical properties of silk scaffolds to be controlled. For example, the molecular weight of the silk protein can be easily altered based on the time spent boiling an original silk solution. Also, the concentration of the silk solution can change the mechanical properties of the synthesized scaffold. Overall, silk is a very versatile biomaterial suitable to be used for tissue engineering (Rnjak-Kovacina 2015).

Collagen

Collagen is one of the most common biomaterials currently used for creating scaffolds. Collagen is the most common protein in extracellular matrices. To date, 28 different types of collagen have been found. Of these 28 different types, Collagen type 1 has been most commonly used for creating synthetic scaffolds. Firstly, since it is the most abundant protein in natural extracellular matrices, collagen is generally biocompatible with human tissues, reducing the risk of body rejection. Another major advantage of using collagen for scaffolds is that collagen fibers can easily become incorporated into the natural extracellular matrices created by the seeded cells. However, collagen does has its own drawbacks. Its mechanical properties prove to be too flexible for rigid tissue repair. However, this has been solved with other methods. Another important property of collagen that makes it a versatile biomaterial is its ability to mix with many substances fairly well. As a result, the weak and overly-flexible collagen can be strengthened by combining it with other biomaterials. The most common biomaterials combine with collagen are glycosaminoglycans (GAGs). GAGs are long polysaccharides created from the repeated linkages of the same single unit disaccharide. However, these collagen-GAG scaffolds still lack the mechanical integrity similar to that of natural extracellular matrices, and therefore a lot more work needs to be done until a perfect collagen-GAG scaffold is created (Nigam, 2014).

Scaffold Synthesis

The methods used to synthesize scaffolds greatly affect the mechanical properties of the scaffolds. There have been countless techniques used to generate cell scaffolds, such as freeze drying (lyophilization), electrospinning, solvent casting and particulate leaching, and self-assembly. Different synthesis techniques are required, depending on the chemical properties of the biomaterials themselves.

Freeze Drying

Freeze drying, which is also known as lyophilization, is a very common technique used to make solid biomaterial sponges and scaffolds. Freeze drying greatly reduces the temperature of the biomaterial causing it to harden. Then, the pressure inside the lyophilization chamber is greatly reduced, until the water in the solution being lyophilized sublimes into water vapor, leaving the scaffold. This process generates a solid solute scaffold with a highly porous interior complex, which can be used to grow cells.

Electrospinning

Electrospinning takes advantage of nanofibers to create the scaffold. Electrospinning starts with a solution containing the desired biomaterial inside a syringe. A tiny drop of the solution is carefully squeezed out until a drop forms on the tip of the needle. This requires that the solution have a fairly high surface tension for the drop to form. Once the drop is formed, a high voltage current is passed through the needle. The drop then slowly starts to form into a cone shape, with the tip of the cone pointing away from the syringe. As the voltage is increased, the tip of the cone gets thinner and longer, creating a microfiber. Microfibers are then collected on a rotating apparatus. Overlaying the nanofibers creates a highly porous and complex scaffold for the cells to grow in. Electrospinning techniques create scaffolds most similar to natural extracellular matrices (Vasita 2006).

Solvent Casting and Particulate Leaching

For solvent casting and particulate leaching, a solution is made containing the biomaterial and a soluble particle, such as salt (NaCl). Once the particles are all fully dissolved, the new solution is poured into a cast of the required shape. Then, the solution is hardened, causing the solvent and the particulates to solidify. Next, the entire scaffold is thoroughly rinsed to leach the particulates out. This method is one of the most commonly used methods for generating scaffolds, because it is the cheapest and requires little expensive technology.

Conclusion

Many different properties of cellular scaffolds affect the regeneration rates of cells in the healing tissue. These properties include the biomaterial composition, such as silk or collagen, which accounts for features such as the biodegradability and biocompatibility of the scaffolds, and the method of synthesis, which changes key properties such as the porosity of the scaffold. Although there are a wide variety of options for scaffold synthesis, no "best" option has been created for medical use. Finding the best scaffolds for particular uses is still a problem being researched+ in the field of tissue engineering. Overall, tissue engineering is a growing field with many unexplored and potentially groundbreaking discoveries.

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